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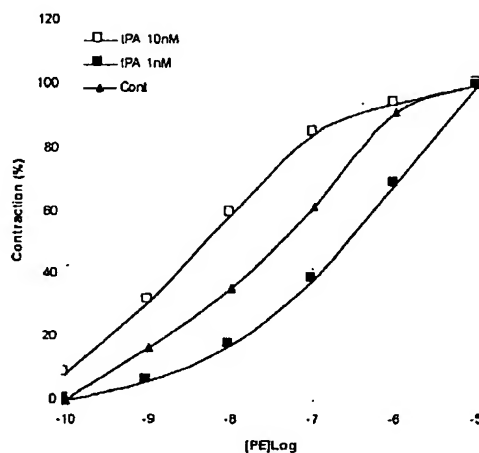
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(54) Title: **PEPTIDE FOR REGULATION OF TISSUE PLASMINOGEN ACTIVATOR**



The effect of tPA on PE induced contraction of isolated rats aorta rings: The contraction of the aorta rings was induced by increasing concentrations of phenylephrine (PE) in the absence of tPA (full triangles) or in the presence of 1 nM (Full squares) or 10 nM tPA (empty squares). The experiments were performed as described by us previously ([5]).

(57) Abstract: The present invention relates to the polypeptide, the use of the polypeptide in the prevention and/or treatment of side effects induced by plasminogen activators, specifically tPA or uPA. The invention further relates to combination compositions and/or therapy regimens, comprising the polypeptide and one or more currently used plasminogen activators, and methods to achieve improved fibrinolytic efficacy as well as reducing their side effects.

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## PEPTIDE FOR REGULATION OF TISSUE PLASMINOGEN ACTIVATOR

### FIELD OF THE INVENTION

This invention discloses a peptide comprising of six amino acids (EEIIMD) having the property to bind at the "docking" site in urokinase plasminogen (uPA) activator and in tissue plasminogen activator (tPA) outside the active site. The invention also relates to the regulation of tPA and uPA activity when tPA or uPA is given in treatment of ischemic stroke, in particular to tPA's capacity to induce intracerebral hemorrhage (ICH).

### BACKGROUND TO THE INVENTION

Tissue-type plasminogen activator is the only therapy for acute thromboembolic stroke, which is approved by the Food and Drug Administration (FDA). However, there is reason for concern that use of tPA for treatment of ischemic stroke may expose patients to secondary intracerebral hemorrhage. *Wardlaw JC et al, Lancet 1997, 350:607-614*. This is because there is an approximately six percent incidence of subsequent symptomatic intracerebral hemorrhage and approximately fifty percent of these patients die. The appearance of intracerebral hemorrhage after treatment with tPA is attributed to its capacity to interfere with the normal vasoactivity of the cerebral blood vessels. TPA has been shown to have dose-dependent vasoconstrictory or vasodilatory effects besides promoting the activation of plasminogen.

Tissue-type plasminogen activator is a naturally occurring molecule released from vascular endothelial cells, and rapid removal of t-PA from the blood occurs by clearance in the liver. Hepatocytes express the low-density lipoprotein receptor-related protein or d<sub>2</sub>-

macroglobulin receptor which bind tPA and complexes of plasminogen activator inhibitor (PAI-1) with tPA and uPA. Alternately, endothelial cells express a 170Kda mannose-dependent receptor which is also involved in the rapid clearance of tPA.

Plasminogen activator inhibitor type 1 interacts with both tPA and uPA and inhibits the catalytic activity of both proteins. PAI-1, which binds tPA and uPA with high affinity is present at high concentrations in the circulation of patients suffering from hypertension. And, reduction of blood pressure by medical treatment results in a decrease of PAI-1 concentrations. The underlying mechanism of action for the increase of PAI-1 in certain pathological conditions is not understood well. However, the inverse relationship with tPA and/or uPA suggests that PAI-1 serves to neutralize in some way the vasoactive effect of tPA and/or uPA. Simmons M, *Cardiol. Clin* 1995, 13:339-345; Cipolla M et al., *Stroke*, 2000, 31:940-945; of PAI-1; and Higazi, A.A.-R et al., *J. Biol. Chem.*, 1997, 272:27053-27057.

The question of whether there is a link between increased levels of PAI-1 concentrations in certain pathological conditions and naturally produced tPA, or whether there is a link between PAI-1 and intracerebral hemorrhage due to use of commercially produced tPA, has not been evaluated heretofore. The present invention is directed to gain a better understanding of the control if any, of PAI-1 or tPA or uPA, and to providing a composition or product optimally effective at regulating activity of tPA or uPA, thereby reducing the risk of intracerebral hemorrhage in patients receiving thrombolytic therapy such as tPA and/or uPA.

SUMMARY OF THE INVENTION

The present invention relates to the composition and use of a polypeptide composed of 6 amino acids () having an inhibitory activity on the vasoactivity of tPA and uPA.

More specifically, the polypeptide is useful in the prevention and/or treatment of hemorrhagic disorders associated with tPA treatment administered for treatment of thromboembolic disorders.

Also, contemplated by the present invention are methods of reducing the occurrence of intracerebral hemorrhage in patients receiving tPA or uPA as fibrinolytic therapy, by adjunctive therapy with .

In yet another embodiment, the present invention is directed to pharmaceutical kits for the treatment of thromboembolic disorders in mammals, the kits comprising a sterile container of tPA in commercially available forms, and a sterile container of each of the agents or a mixture of agents, both in amounts therapeutically effective to treat the thromboembolic disorders, while in the same regimen, preventing the occurrence of side effects of tPA.

The foregoing kits may include, if desired, uPA or tPA in amounts therapeutically effective to treat thromboembolic disorders as well as prevent any side effects.

It is also within the scope of this invention to provide kits of tPA or uPA in combination regimens of other fibrinolytic agents, along with where appropriate. It is further the object of the present invention to provide methods of treating thromboembolic disorders using as conjunctive therapy in combination with one or more of fibrinolytic agents including tPA, uPA, tPA, streptokinase, rt-PA or alteplase, rt-PA derivatives (such as reteplase, lanoteplase and TNK-rt-PA), anisoylated plasminogen streptokinase complex (APSC) or anistreplase, or

### BRIEF DESCRIPTION OF THE FIGURES

The advantages and features of the present invention will become readily apparent after reading the following detailed description and referencing the drawings, which are:

Fig. 1 is a graph describing the effect of tPA on phenylephrine-induced contraction of isolated rat aorta rings in vitro. The contraction of the aorta rings was induced by varying concentrations of phenylephrine in the absence of tPA (filled triangles), in the presence of 1nM of tPA (filled squares) or in the presence of 10nM tPA (empty squares). The experiments were performed according to procedures described earlier by *Haj-Yehia A et al.*, FASEB J, 2000, 14:1411-1422.

Fig. 2 is a bar diagram describing the results of experiments on the vasoactivity of uPA and tPA in the presence or absence of PAI-1, for example, the effect of 2nM uP or 1nM tPA on phenylephrine induced vasoconstriction was determined in the presence or absence of equimolar concentrations of PAI-1.

Fig. 3 is a bar diagram describing the results of studies done on the effect of PAI-1 derived peptide on tPA vasoactivity. The constriction of aorta rings was induced by increasing the concentrations of phenylephrine in the absence or presence of 1nM tPA, 1nM tPA and 1 OM , 10mM tPA or 10nM tPA and 1 OM .

Fig. 4 is a bar diagram describing the results of experiments on the effect of PAI-1 derived peptide on tPA mediated clot lysis. The capacity of tPA to induce clot lysis was determined in the presence and absence of 1OM . In these experiments, blood from volunteers was allowed to clot at room temperature for one hour, the blood clot was separated from the

plasma, placed on absorbing paper to remove all the serum and cut into several pieces. The pieces were weighed, and placed in PBS buffer alone or containing 100 nM tPA, with or without 10M . After incubation for 3 hours at room temperature, the thrombi are separated from the medium, dried and weighed.

### DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, pharmaceutical compositions of the peptide are provided, such compositions having inhibitory effects on tPA and/or uPA related hemorrhagic disorders that result as serious side effects of such fibrinolytic agents. Also, contemplated by the present invention are methods of reducing the occurrence of intra-cerebral hemorrhage in patients receiving tPA or uPA in the treatment of thromboembolic disorders.

The present invention also provides pharmaceutical compositions and kits comprising of the polypeptide in combination with one or more of fibrinolytic agents including tPA, uPA, scuPA, tcuPA, streptokinase, rt-PA or alteplase, rt-PA derivatives (such as reteplase, lanoteplase and TNK-rt-PA), anisoylated plasminogen streptokinase complex (APSC) or anistreplase, or streptokinase derivatives.

The present invention further provides methods for preventing and/or treating side effects such as intracerebral hemorrhage and related vascular abnormalities associated with fibrinolytic agents such as tPA or uPA, by providing therapeutic regimens – solo or in combination, in combination with an effective amount of to prevent and/or inhibit side effects.

TPA is a single-chain serine protease composed of 530 amino acids, although originally 527 were identified. The t-PA enzyme is composed of several domains with homologies to other proteins:

A finger domain comprising residues 4-50,

A growth factor domain comprising residues 50-87,

two kringles comprising residues 87-176 and 176-262, and the protease domain

constituted by residues 276-527 comprising the catalytic triad. Initial binding of t-PA to fibrin is governed by the finger domain and by kringle 2, which binds to exposed carboxyl-terminal lysine residues.

TPA has a weak affinity for plasminogen in the absence of fibrin ( $K_m = 76 \mu M$ ) but a much higher affinity in the presence of fibrin ( $K$  between 0.15 and 1.5  $\mu M$ ). In this reaction plasminogen binds to fibrin primarily via specific structures called the "lysine-binding site." Thus one way of regulating fibrinolysis is at the level of plasminogen activation localized at the fibrin surface.

Plasminogen activator inhibitors, specifically PAI-1 and PAI-2 inhibit the physiological plasminogen activators, for example, PAI-1 is the primary inhibitor of t-PA and u-PA in plasma. PAI-1, a serine protease inhibitor, is a single chain glycoprotein derived from endothelial cells and other cell types. PAI-1 inhibits tPA by the formation of a complex between the active site of tPA and the "bait" residues (Arg 346-Met 347) of PAI-1.

The PAI-1 concentration in plasma is increased in several diseases, including venous thromboembolism, obesity, sepsis and coronary artery disease. High PAI-1 activity constitutes an independent risk factor for myocardial infarction in young subjects within three (3) years of the first attack. There is a clear correlation between the circadian variation in the time of onset of myocardial infarction, with the highest incidence at about 8 am and the circadian rhythm of plasma PAI-1 activity which is also highest early in the morning.



Plasminogen activator inhibitor type 1 interacts with both tPA and uPA and inhibits the catalytic activity of both proteins. PAI-1, which binds to tPA and uPA with high affinity (Heckman CM, Archives of Biochem Biophysics, 1988, 262:199-210), is also present at high concentrations in the circulation of patients suffering from hypertension. Reduction of blood pressure by medical treatment results in the decrease of PAI-1 concentration. Erden YC *et al. AmJ Hypertens*, 1999, 12:1071-1076. The underlying mechanism of action to explain the increase of PAI-1 in some pathological conditions is not understood.

PAI-1 reacts with single chain tPA, two chain tPA and tPA. The second-order rate constant for their inhibition of single-chain tPA by PAI-1 is about  $10^7 \text{ M}^{-1} \text{ s}^{-1}$ , while inhibition of two chain tPA and tPA is somewhat faster. Positively charged regions in tPA (residues 296-304) and uPA (residues 179-184) are involved in this rapid reaction. PAI activity is very rapidly cleared from the circulation by the liver. Except for platelets, which contain both functional and inactive PAI-1, PAI-1 is not stored within cells, but is rapidly and constitutively secreted after synthesis.

PAI-1 binds tPA and uPA through two independent epitopes, one of which interacts with the active site. The other epitope is composed of 6 amino acid residues, EEIMD, that correspond to the amino acid residues 350 to 355 of PAI-1. This second epitope of PAI-1 interacts with a binding "docking" site in uPA and tPA that is outside of the active site. Adams DS *et al., J. Biol. Chem*, 1999, 266:8476-8482.

The present invention describes the effect of the peptide on the vasoactivity of tPA and uPA and indicates that the peptide abolishes the enhancing effect of tPA on phenylephrine-induced vasoconstriction in aorta ring cultures. Similarly, the peptide of the present invention

abrogates the enhancing effect of uPA on phenylephrine-induced vasoconstriction. These observations are described in detail in the Examples section.

The peptide of the present invention, while preventing and/or inhibiting the adverse effects of tPA or uPA on blood vessels has no effect on the fibrinolytic activity of tPA or uPA, so useful in clot lysis during thrombolytic therapy in myocardial infarction, stroke and related complications.

The commercially available tPA is produced by recombinant DNA technology (such as recombinant t-PA, rt-PA) in two forms: a single-chain preparation (alteplase) and a double-chain preparation (dute plase). Other tPA types include reteplase (r-PA0 and a mutant of rt-PA, TNK-rt-PA.

The preferred dosage regimen of fibrin-selective alteplase consists of a weight-adjusted accelerated (front-loaded) regimen over 90 minutes (15 mg bolus, 0.75 mg/kg over 30 minutes (not to exceed 50 mg] and .05 mg/kg over 60 minutes [not to exceed 35 mg]).

The preferred dosage regimen for the peptide consists of an amount effective to prevent the harmful vasoactive effects of tPA on a case by case basis. The peptide may be a component of a sequence of varying numbers of amino acids, or the peptide may have a modification of one or more amino acids in its sequence.

The peptide of the present invention is useful in treatment of sepsis, when administered alone in an effective dosage or in combination with traditional anti-coagulant therapy. Under physiological conditions, several antithrombotic mechanisms act in concert to prevent clotting, and to preserve blood fluidity. Any thrombin that escapes the surveillance of this physiological anticoagulant system is available to convert fibrinogen to fibrin. This in turn triggers the

fibrinolytic system.

## EXAMPLES

### Effect of tPA on Phenylephrine Induced Contraction

Fig. 1 describes a graph describing the effect of tPA on phenylephrine-induced contraction of isolated rat aorta rings in vitro. The contraction of the aorta rings was induced by varying concentrations of phenylephrine in the absence of tPA (filled triangles), in the presence of 1nM of tPA (filled squares) or in the presence of 10nM tPA (empty squares). The experiments were performed according to procedures described earlier by *Haj-Yehia A et al.*, FASEB J, 2000, 14:1411-1422.

Results obtained confirm that tPA has the capacity to induce vasodilatation.

Fig. 1 shows that the presence of 1nM tPA inhibits the vasoconstriction induced by phenylephrine. Increased tPA concentrations induced the opposite effect, i.e., the presence of 10nM tPA stimulated the vasoconstriction induced by phenylephrine. Similarly uPA has the capacity to induce vasoconstriction (*Haj-Yehia A., et al. FASEB J*, 2000, 14:1411-1422).

### Effect of PAI-1 on Vasoactivity of uPA and tPA

Fig. 2 describes a bar diagram describing the results of experiments on the vasoactivity of uPA and tPA in the presence or absence of PAI-1, for example, the effect of 2nM uPA or 1nM tPA on phenylephrine induced vasoconstriction was determined in the presence or absence of equimolar concentrations of PAI-1.

Fig. 3 is a bar diagram describing the results of studies done on the effect of PAI-1 derived peptide on tPA vasoactivity. The constriction of aorta rings was induced by increasing the concentrations of phenylephrine in the absence or presence of 1nM tPA, 1nM tPA and 1

OM, 10 nM tPA or 10nM tPA and 1 OM .

Results obtained show that 1 OM of abolished the enhancing effect of tPA on phenylephrine induced vasoconstriction. exerted the same effect on uPA. Neither PAI-1 nor alone had any effect on contraction of aorta rings Fig. 3. Therefore, the mechanism through which PAI-1 affects the vasoactive effect of tPA or uPA is through its interaction with the docking site.

#### Effect of PAI-1 derived peptide on tPA medicated clot lysis

Fig. 4 is a bar diagram describing the results of experiments on the effect of PAI-1 derived peptide on tPA mediated clot lysis. The capacity of tPA to induce clot lysis was determined in the presence and absence of 10M . In these experiments, blood from volunteers was allowed to clot at room temperature for one hour, the blood clot was separated from the plasma, placed on absorbing paper to remove all the serum and cut into several pieces. The pieces were weighed, and placed in PBS buffer alone or containing 100 nM tPA, with or without 10M . After incubation for 3 hours at room temperature, the thrombi are separated from the medium, dried and weighed.

Two methods were used to determine whether the peptide affected the fibrinolytic activity of tPA by inhibiting plasminogen activity: 1) The chromogenic assay described in detail earlier (*Higazi AA.-R, et al. J. Biol. Chem.*, 1995, 270:9472-9477); and 2) The clots lysis test described earlier (*Higazi AA-R et al., Blood* 1988, 92:2075-2083).

Results obtained show that had no significant effect on the catalytic activity of the tPA.

Fig. 4

Therefore, these data indicate that the PAI-1 derived peptide and its derivatives can neutralize the vasoactivity of tPA or uPA, thereby reducing their adverse effects on blood vessels and preventing the complications that appear during thrombolytic therapy as in the case of myocardial infarction, stroke and similar diseases.

The present invention is not to be limited in scope by the embodiment disclosed in the example which is intended as an illustration of one aspect of the invention and any methods which are functionally equivalent are within the scope of the invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, any equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the claims.

What is claimed is:

1. A polypeptide, comprising six amino acids, said polypeptide having an inhibitory effect on vasoactivity induced by plasminogen activators.
2. The polypeptide according to claim 1, said polypeptide being a component of a sequence of varying numbers of amino acids, or said polypeptide having a modification of one or more amino acids in its sequence.
3. The polypeptide according to claim 1, wherein the plasminogen activator includes tPA or uPA.
4. The peptide according to claim 1, wherein the plasminogen activator includes tPA, streptokinase, rt-PA, rt-PA derivatives, APSC, recombinant scuPA prourokinase or the covalent cross linked scuPA/suPAR complex .
5. The polypeptide according to claim 1, further including tPA and said combination having fibrinolytic activity without causing hemorrhage.
6. The polypeptide according to claim 1, further including uPA, said combination having fibrinolytic activity without causing hemorrhage.
7. The polypeptide according to claim 1, further including one or more plasminogen activators including tPA, streptokinase, rt-PA, rt-PA derivatives, APSC, recombinant scuPA prourokinase or the covalent cross linked scuPA/suPAR complex, said combination having fibrinolytic activity.
8. A method of fibrinolytic therapy in a patient in need thereof, said method comprising administering to the patient a thrombolytic dosage of a thrombolytic agent and thereafter administering an effective supplemental dosage of EEIIMI in

an amount that prevents hemorrhage or side effects, said supplemental dosage of EEIIMI being administered once every 1 to 10 days for the duration of the therapy.

9. The method of fibrinolytic therapy according to claim 8, wherein the thromolytic agent includes tPA or uPA and is administered at a standard clinical thrombolytic agent and a sufficient dosage of .
10. The method of fibrinolytic therapy according to claim 8, wherein the supplemental dosage of is a bolus up to of 500 mg. .
11. A method of preventing hemorrhage in a patient receiving said method comprising administering to the patient once every 1 to 10 days a bolus of an amount of , wherein said subsequently inhibits vasoactive effects of plasminogen activators including tPA or uPA given at standard clinical dosages.
12. The method of fibrinolytic therapy according to claim 8, wherein the thrombolytic agent is tPA or scuPA.
13. The method of fibrinolytic therapy according to claim 8, further comprising one or more of plasminogen activators essentially comprising of one or more of the plasminogen activators essentially comprising of tPA, streptokinase, rt-PA, rt-PA derivatives, APSC, recombinant scuPA prourokinase or the covalent cross linked scuPA/suPAR complex.

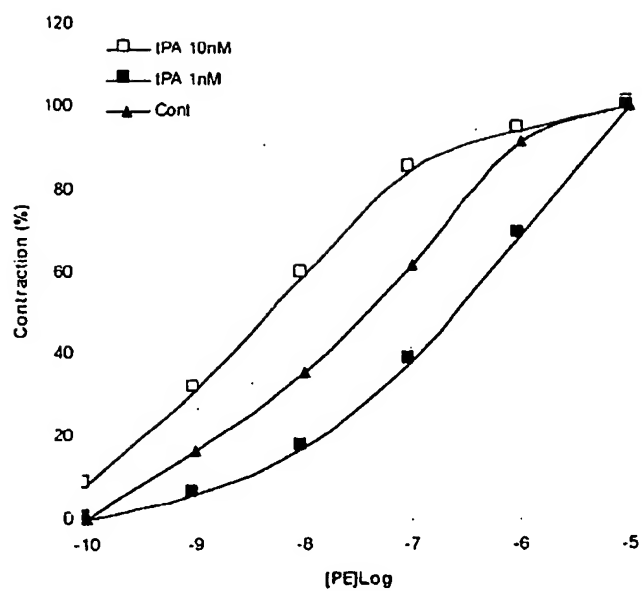


Figure 1: The effect of tPA on PE induced contraction of isolated rats aorta rings: The contraction of the aorta rings was induced by increasing concentrations of phenylephrine (PE) in the absence of tPA (full triangles) or in the presence of 1 nM (Full squares) or 10 nM tPA (empty squares). The experiments were performed as described by us previously ([5]).



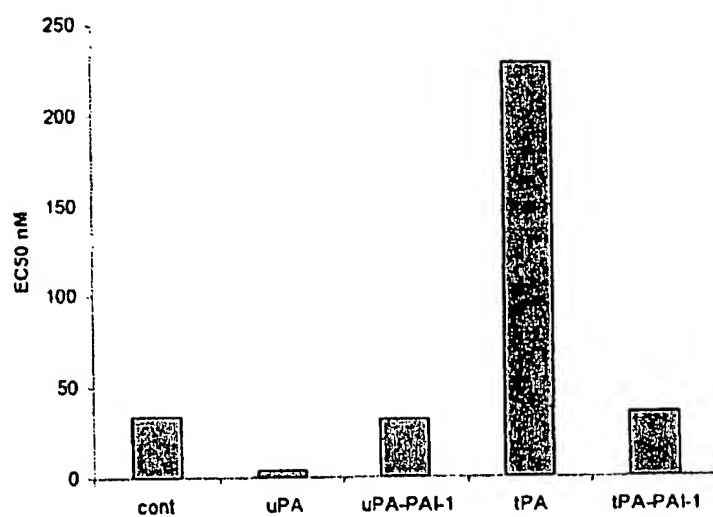


Figure 2: The effect of PAI-1 on the vasoactivity of uPA and tPA. The effect of 2 nM uPA or 1 nM tPA on PE induced vasoconstriction was determined in the presence or absence of equimolar concentrations of PAI-1

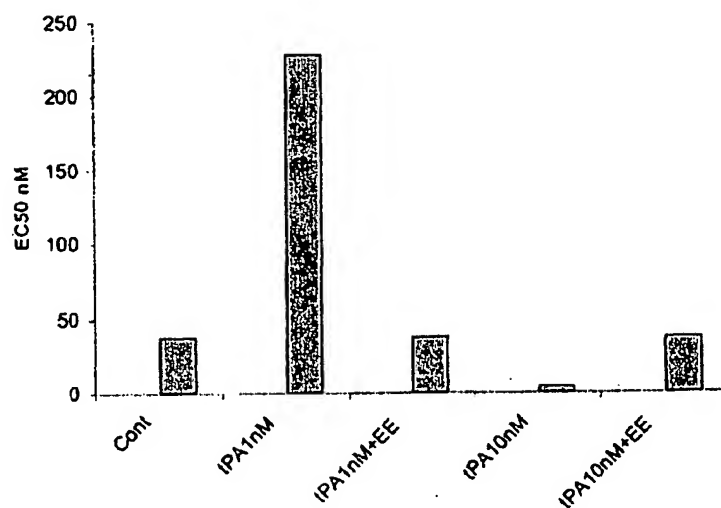


Figure 3: The effect of PAI-1 derived peptide on tPA vasoactivity. The constriction of aorta rings was induced by increasing the concentrations of PE (as in Fig.1) in the absence (control) or presence of 1 nM tPA, 1 nM IPA and 1  $\mu$ M EEIIMD, 10 nM tPA or 10 nM IPA and 1  $\mu$ M EEIIMD.

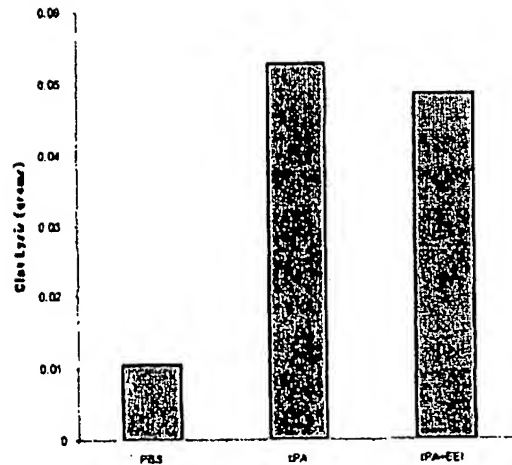


Figure 4. The effect of the PAI-1 derived peptide on tPA mediated clot lysis. The capacity of tPA to induce clot lysis was determined in the presence and absence of 1  $\mu$ M EEIIMD. In these experiments, blood was taken from volunteers: one hour after clotting at room temperature, the blood clot was separated from the plasma, placed on absorbing paper to remove all the serum and cut into several pieces. The pieces were weighed, and placed in PBS buffer alone or containing 100 nM tPA, without or with 1  $\mu$ M EEIIMD. After incubation for 3 hours at room temperature, the thrombi were separated from the medium, dried and weighed.

## INTERNATIONAL SEARCH REPORT

International application No.  
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<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(7) :A61K 38/00, 38/08 US CL :514/2; 530/350, 380; 980/10, 250 According to International Patent Classification (IPC) or to both national classification and IPC														
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/2; 530/350, 380; 980/10, 250 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Extra Sheet.														
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>														
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
X	ZHANG et al. Regulation of Single Chain Urokinase Binding Internalization, and Degradation by a Plasminogen Activator Inhibitor 1-Derived Peptide, J. Biol. Chem. October 1997, Vol. 272, No. 43, pp. 27053-27057, see entire document.	1-7												
X	XUE et al. Interfering with the inhibitory mechanism of serpins: crystal structure of a complex formed between cleaved plasminogen activator inhibitor type 1 and a reactive-centre loop peptide, Structure, 1998, Vol. 6, No. 5, pp. 627-636, see especially sequence.	1-7												
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.														
<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"E" earlier document published on or after the international filing date</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"A" document member of the same patent family</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td></td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A" document member of the same patent family	"O" document referring to an oral disclosure, use, exhibition or other means		"P" document published prior to the international filing date but later than the priority date claimed	
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"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art													
"I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A" document member of the same patent family													
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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US02/20077

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ADAMS et al. A synthetic DNA encoding a modified human urokinase resistant to inhibition by serum plasminogen activator inhibitor, J. Biol. Chem. May 1991, Vol. 266, No. 13, pp. 8476-8482, see especially last five lines of abstract and p. 8481, Col. 1, Discussion.	1-13
A, P	CIPOLLA et al. Postischemic Attenuation of Cerebral Artery Reactivity is Increased in the Presence of Tissue Plasminogen Activator, Stroke, April 2000, Vol. 31, No. 4, pp. 940-945, see entire document.	1-13
A, P	HALEY, EC. Editorial Comment: Postischemic attenuation of cerebral artery reactivity is increased in the presence of tissue plasminogen activator, Stroke, April 2000, Vol. 31, No. 4, page 945, see especially first sentence second paragraph.	1-13

## INTERNATIONAL SEARCH REPORT

International application No.

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### B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

STN (Bioscience); EAST (all databases); search terms: EEIIMD, EEIIMI, fibrinolytic, vasoconstrict?, inh? vasoactivity, tctPA, tPA, streptokinase, SK, alteplase, rt-PA, reteplase, anisoylated plasminogen streptokinase complex or APSC, rec scuPA, prouPA, scuPA/suPAR, anistreplase, hemorrhage, thrombolytic